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Atty. Docket No.: 25436/2152 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Holly Hogrefe, et al.	Examiner:	Not Yet Assigned
Serial No.:	10/035,091		
Filed:	December 21, 2001	Group Art Unit:	Not Yet Assigned
Entitled:	High Fidelity DNA Polymerase Compositions and Uses Therefor	Conf. No.:	1719

Box: Missing Parts
Commissioner for Patents
Washington, D.C. 20231

RESPONSE TO NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE AND/OR AMINO ACID SEQUENCE DISCLOSURES

Sir:

A sequence listing is submitted in response to the Notice to File Missing Parts mailed February 8, 2002.

In the originally filed specification, please enter the following amendment:

1. Please replace the first full paragraph on page 21 with the following paragraph:

"The ϕ29 DNA polymerase mutagenesis studies targeted amino acid residues within highly conserved Family B motifs (DXXSLYP [SEQ ID NO. 1], KXXXNSXYG [SEQ ID NO. 2], TXXGR [SEQ ID NO. 3], YXDTDS [SEQ ID NO. 4], and KXY [SEQ ID NO. 5]), although other regions of the protein presumably can be mutagenized to selectively decrease DNA polymerase activity. One such region is the partitioning domain, characterized by the YXGG [SEQ ID NO. 6] motif (Truniger et al., 1996, EMBO J. 15:3430-3441). This region is located within an accessible loop connecting the 3'-5' exonuclease and polymerase domains. The partitioning domain plays a critical role in coordinating the balance between synthesis and degradation of the DNA chain. Mutations within this region disrupt the equilibrium between polymerization and proofreading, and produce phenotypes favoring either polymerization (reduced proofreading) or proofreading (reduced polymerization)."

2. Please replace the last paragraph on page 21 (continued onto page 22) of the originally filed application with the following paragraph:

"In one embodiment of the invention, Pfu DNA polymerase was mutated within the partitioning domain at amino acids 384-389 (SYTGGF [SEQ ID NO. 7]) to obtain a Pfu DNA polymerase with reduced polymerization activity. It is understood that other amino acid side substitutions within the partitioning domain, e.g., at positions Y385, G387, G388, could also selectively reduce DNA polymerase activity while having minimal effects on exonuclease activity."

3. Please replace the last paragraph on page 57 (continued to page 58) with the following paragraph:

"A qualitative assay was used to verify that Pfu mutants retained 3'-5' exonuclease activity under PCR conditions. In this assay, the 900bp Hα1AT target is amplified with exo$^-$ Pfu DNA polymerase (2.5U/50µl) using a forward primer containing a 3'dG, which produces a dG/dG mismatch upon annealing to the DNA template. The amplicon is amplified from humna genomic DNA using the forward primer: 5'-GAG.GAG.AGC.AGG.AAA.GGT.GGA.A**G**-3' [SEQ ID NO. 8] (100ng/50µl rxn) and the reverse primer: 5'-GAG.GTA.CAG.GGT.TGA.GGC.TACT.G – 3' [SEQ ID NO. 9] (100ng/50µl rxn). Amplification is carried out in the absence or presence of varying amounts of Pfu mutants (200ng to 3.6µg) on a Perkin/Elmer 9600 thermal cycler with the following program: (1 cycle) 95°C for 2.5 minutes; (30 cycles) 95°C for 40 seconds, 61°C for 10 seconds, 72°C for 2.5 minutes; (1 cycle) 72°C for 7 minutes. In the absence of proofreading activity, exo$^-$ Pfu produces low yields of product, presumably because the enzyme can not efficiently extend a dG/dG mismatch. In the presence of Pfu mutants with proofreading activity, the 3'dG should be excised from the primer, thereby allowing exo$^-$ Pfu to amplify the target in high yields. This PCR assay was used to verify that Pfu mutants tested in fidelity assays retained sufficient proofreading activity under PCR conditions to excise mismatched PCR primers. Moreover, the assay allowed us to determine the range of protein concentrations that could be added to PCR reactions without inhibition of amplification."

Marked-up versions for the amended paragraphs are enclosed.

Applicants, in compliance with 37 C.F.R. 1.821(f), hereby state that the information recorded in computer readable form submitted herewith is identical to the written sequence listing. Applicants, in compliance with 37 C.F.R. 1.821(g), further state that no new matter is added.

Respectfully submitted,

Date: 4/8/02

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Marked-up version **Atty. Docket No.: 25436/2152**

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